

SEQUENCE LISTING

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Hegedus, Dwayne
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<120> A Repressor-Mediated Regulation System for Control of Gene Expression
in Plants

<130> 1096.021A

<150> PCT/CA02/00740

<151> 2002-05-23

<150> US 60/292,973

<151> 2001-05-23

<160> 44

<170> PatentIn version 3.0

<210> 1

<211> 429

<212> DNA

<213> Agrobacterium tumefaciens

<400> 1
atgacggaaa ctgcatacgg taacgcccag gatctgctgg tcgaactgac ggcggatatt 60
gtggctgcct atgttagcaa ccacgtcggt cgggtaactg agcttcccgg ccttatttcg 120
gatgttcata cggcactcag cggaacatcg gcaccggcat cggtgggcgt caatgttgaa 180
aagcagaagc ctgctgtgtc ggttcgcaag tcggttcagg acgatcatat cgtctgtttg 240
gaatgtggtg gtcggttcaa gtcgctcaaa cgccacctga cgacgcatca cagcatgacg 300

ccggaagaat atcgcgaaaa atgggatctg ccggtcgatt atccgatggt tgctcccgcc 360
tatgccgaag cccgttcgcg gctcgccaag gaaatgggtc tcggtcagcg ccgcaaggcg 420
aaccgttga 429

<210> 2

<211> 458

<212> DNA

<213> Artificial

<220>

<223> synthetic ROS optimized for plant codon usage and encoding fusion
of ROS and nuclear localization signa

<400> 2

atgactgaga ctgcttacgg taacgctcag gatcttcttg ttgagcttac tgctgatatc 60
gttgctgctt acgtttctaa ccacgttggt cctgttactg agcttcctgg acttatctct 120
gatgttcata ctgcactttc tggaacatct gtcctgctt ctggtgctgt taacgttgag 180
aagcagaagc ctgctgtttc tgttcgtaag tctgttcagg atgatcatat cgtttgtttg 240
gagtgtggtg gttctttcaa gtctctcaag cgtcacctta ctactcatca ctctatgact 300
ccagaggagt atagagagaa gtgggatctt cctgttgatt accctatggt tgctcctgct 360
tacgctgagg ctcgttctcg tctcgctaag gagatgggtc tcggtcagcg tcgtaaggct 420
aaccgtccaa aaaagaagcg taaggctctga gagctcgc 458

<210> 3

<211> 447

<212> DNA

<213> Artificial

<220>

<223> ROS consensus sequence

<220>

<221> misc_feature
<222> (1)..(447)
<223> n is A or T or G or C

<220>

<221> misc_feature
<222> (1)..(447)
<223> h is a or c or t/u

<220>

<221> misc_feature
<222> (1)..(447)
<223> m is a or c

<220>

<221> misc_feature
<222> (1)..(447)
<223> r is G or A

<220>

<221> misc_feature
<222> (1)..(447)
<223> y is t/u or c

<220>

<221> misc_feature
<222> (1)..(447)
<223> w is a or t/u

<400> 3
atgacngara cngcntaygg naaygcncar gayytnytng tngarytnac ngcngayath 60
gtngcngcnt aygtnewsnaa ycaygtngtn ccngtnacng arytnccngg nytnathwsn 120
gaygtncaya cngcnytnws nggnacnwsn gcncncngcnw sngtngcngt naaygtngar 180
aarcaraarc cngcngtnws ngtnmgnaar wsngtncarg aygaycayat hgtntgyytn 240
gartgyggng gnwsnttyaa rwsnytnaar mgncayytna cnacncayca ywsnatgacn 300
ccngargart aymngarara rtggggyytn ccngtngayt ayccnatggt ngcncngcn 360
taygcngarg cnmgnewsnmg nytngcnaar garatgggny tnggncarmg nmgnaargcn 420
aaymgncna araaraarmg naargtn 447

<210> 4

<211> 27

<212> DNA

<213> Artificial

<220>

<223> sense primer for amplifying ROS coding region

<220>

<221> misc_feature

<222> (60)..(447)

<223> where n is A or T or G or C

<400> 4
gcggatccga tgacggaaac tgcatac 27

<210> 5

<211> 25

<212> DNA

<213> Artificial

<220>

<223> antisense primer for amplifying ROS coding region

<400> 5

gcaagcttca acggttcgcc ttgcg

25

<210> 6

<211> 36

<212> DNA

<213> Artificial

<220>

<223> sense primer for amplifying tms2 promoter

<400> 6

tgcggatgca taagcttgct gacattgcta gaaaag

36

<210> 7

<211> 26

<212> DNA

<213> Artificial

<220>

<223> antisense primer for amplifying tms2 promoter

<400> 7

cggggatcct ttcagggcca tttcag

26

<210> 8

<211> 25

<212> DNA

<213> Artificial

<220>

<223> ROS operator sequence

<400> 8
tatatttcaa ttttattgta atata 25

<210> 9

<211> 109

<212> DNA

<213> Artificial

<220>

<223> ROS-OPDS oligo for p74-315 construct

<400> 9
atctccactg acgtaaggga tgacgcacaa tcccactatc cttegcaaga cccttcctct 60
atataatata tttcaatttt attgtaatat aacacggggg actctagag 109

<210> 10

<211> 113

<212> DNA

<213> Artificial

<220>

<223> ROS-OPDA oligo for p74-315 construct

<400> 10
gatcctctag agtccccgt gttatattac aataaaattg aaatatatta tatagaggaa 60
gggtcttgcg aaggatagtg ggattgtgcg tcatccctta cgtcagtgga gat 113

<210> 11

<211> 107

<212> DNA

<213> Artificial

<220>

<223> ROS-OPUS oligo for p74-316 construct

<400> 11
atctccactg acgtaaggga tgacgcacaa tctatatattc aattttattg taatatacta 60
tataaggaag ttcatttcat ttggagagaa cacgggggac tctagag 107

<210> 12

<211> 111

<212> DNA

<213> Artificial

<220>

<223> ROS-OPUA oligo for p74-316 construct

<400> 12
gatcctctag agtcccccggt gttctctcca aatgaaatga acttccttat atagtatatt 60
acaataaaaat tgaaatatag attgtgctgc atcccttacg tcagtggaga t 111

<210> 13

<211> 108

<212> DNA

<213> Artificial

<220>

<223> ROS-OPPS oligo for p74-309 construct

<400> 13
atctccactg acgtaaggga tgacgcacaa tctatatattc aattttattg taatatacta 60
tataatatat ttcaatttta ttgtaatata acacggggga ctctagag 108

<210> 14

<211> 112

<212> DNA

<213> Artificial

<220>

<223> ROS-OPPA oligo for p74-309 construct

<400> 14

gatcctcttag agtcccccggt gttatattac aataaaaattg aaatatatta tatagtatat 60

tacaataaaaa ttgaaatata gattgtgcgt catcccttac gtcagtggag at 112

<210> 15

<211> 59

<212> DNA

<213> Artificial

<220>

<223> ROS-OP1 oligo for p76-508 construct

<400> 15

gatcctatat ttcaatttta ttgtaatat gctatatattc aattttattg taatataat 59

<210> 16

<211> 57

<212> DNA

<213> Artificial

<220>

<223> ROS-OP2 oligo for p76-508 construct

<400> 16

cgatttatatt acaataaaaat tgaaatatag ctatattaca ataaaattga aatatag 57

<210> 17

<211> 24

<212> DNA

<213> Artificial

<220>

<223> sense primer for amplifying Actin2 promoter

<400> 17

aagcttatgt atgcaagagt cagc

24

<210> 18

<211> 24

<212> DNA

<213> Artificial

<220>

<223> antisense primer for amplifying Actin2 promoter

<400> 18

ttgactagta tcagcctcag ccat

24

<210> 19

<211> 27

<212> DNA

<213> Artificial

<220>

<223> ROS operator sequence in ipt gene

<400> 19

tataattaaa atattaactg tcgcatt

27

<210> 20

<211> 10

<212> DNA

<213> Artificial

<220>

<223> consensus ROS operator sequence

<400> 20
watdhwkmar

10

<210> 21

<211> 104

<212> PRT

<213> Agrobacterium radiobacter

<400> 21

Met Thr Thr Ala Tyr Gly Asn Ala Asp Val Thr Ala Asp Val Ala Ala
1 5 10 15

Tyr Val Ser Asn His Val Val Val Thr Gly Ser Asp Val His Thr Ala
20 25 30

Ser Gly Thr Ser Ala Ala Ser Val Ala Val Asn Val Lys Lys Ala Val
35 40 45

Ser Val Arg Lys Ser Val Asp Asp His Val Cys Cys Gly Gly Ser Lys
50 55 60

Ser Lys Arg His Thr Thr His His Ser Met Thr Tyr Arg Lys Trp Asp
65 70 75 80

Val Asp Tyr Met Val Ala Ala Tyr Ala Ala Arg Ser Arg Ala Lys Met
85 90 95

Gly Gly Arg Arg Lys Ala Asn Arg
100

<210> 22

<211> 138

<212> DNA

<213> Artificial

<220>

<223> p74-315 sequence from EcoRV site to ATG codon of GUS

<400> 22
gatatctcca ctgacgtaag ggatgacgca caatcccact atccttcgca agacccttcc 60

tctatataat atatttcaat tttattgtaa tataacacgg gggactctag aggatccccg 120

ggtggtcagt cccttatg 138

<210> 23

<211> 136

<212> DNA

<213> Artificial

<220>

<223> p74-316 sequence from EcoRV site to ATG codon of GUS

<400> 23
gatatctcca ctgacgtaag ggatgacgca caatctatat ttcaatttta ttgtaatata 60

ctatataagg aagttcattt catttggaga gaacacgggg gactctagag gatccccggg 120

tggtcagtc cttatg 136

<210> 24

<211> 137

<212> DNA

<213> Artificial

<220>

<223> p74,309 sequence from EcoRV site to ATG codon of GUS

<400> 24
gatatctcca ctgacgtaag ggatgacgca caatctatat ttcaatttta ttgtaatata 60

ctatataata tatttcaatt ttattgtaat ataacacggg ggactctaga ggatccccgg 120

gtggtcagtc ccttatg 137

<210> 25

<211> 237

<212> DNA

<213> Artificial

<220>

<223> p74-118' sequence from EcoRV site to ATG codon of GUS

<400> 25

gatatctcca ctgacgtaag ggatgacgca caatcccact atccttcgca agacccttcc 60
tctatataat atatttcaat tttattgtaa tataacacgg gggactctag aggatcctat 120
atttcaattt tattgtaata tagctatatt tcaattttat tgtaatataa tcgatttcga 180
acccggggta ccgaattcct cgagtctaga ggatccccgg gtggtcagtc ccttatg 237

<210> 26

<211> 142

<212> PRT

<213> Agrobacterium tumefaciens

<400> 26

| | | | | | | | | | | | | | | | |
|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| Met | Thr | Glu | Thr | Ala | Tyr | Gly | Asn | Ala | Gln | Asp | Leu | Leu | Val | Glu | Leu |
| 1 | | | | 5 | | | | 10 | | | | | 15 | | |
| Thr | Ala | Asp | Ile | Val | Ala | Ala | Tyr | Val | Ser | Asn | His | Val | Val | Pro | Val |
| | | | 20 | | | | 25 | | | | | 30 | | | |
| Thr | Glu | Leu | Pro | Gly | Leu | Ile | Ser | Asp | Val | His | Thr | Ala | Leu | Ser | Gly |
| | | 35 | | | | 40 | | | | | 45 | | | | |
| Thr | Ser | Ala | Pro | Ala | Ser | Val | Ala | Val | Asn | Val | Glu | Lys | Gln | Lys | Pro |
| | 50 | | | | 55 | | | | | 60 | | | | | |
| Ala | Val | Ser | Val | Arg | Lys | Ser | Val | Gln | Asp | Asp | His | Ile | Val | Cys | Leu |
| 65 | | | | 70 | | | | 75 | | | | | | 80 | |
| Glu | Cys | Gly | Gly | Ser | Phe | Lys | Ser | Leu | Lys | Arg | His | Leu | Thr | Thr | His |
| | | | 85 | | | | | 90 | | | | | 95 | | |
| His | Ser | Met | Thr | Pro | Glu | Glu | Tyr | Arg | Glu | Lys | Trp | Asp | Leu | Pro | Val |
| | | 100 | | | | | 105 | | | | | 110 | | | |
| Asp | Tyr | Pro | Met | Val | Ala | Pro | Ala | Tyr | Ala | Glu | Ala | Arg | Ser | Arg | Leu |
| | 115 | | | | | 120 | | | | | 125 | | | | |
| Ala | Lys | Glu | Met | Gly | Leu | Gly | Gln | Arg | Arg | Lys | Ala | Asn | Arg | | |
| | 130 | | | | 135 | | | | | | 140 | | | | |

<210> 27

<211> 149

<212> PRT

<213> Artificial

<220>

<223> synthetic-ROS fused to nuclear localization signal

<400> 27

Met Thr Glu Thr Ala Tyr Gly Asn Ala Gln Asp Leu Leu Val Glu Leu
1 5 10 15
Thr Ala Asp Ile Val Ala Ala Tyr Val Ser Asn His Val Val Pro Val
20 25 30
Thr Glu Leu Pro Gly Leu Ile Ser Asp Val His Thr Ala Leu Ser Gly
35 40 45
Thr Ser Ala Pro Ala Ser Val Ala Val Asn Val Glu Lys Gln Lys Pro
50 55 60
Ala Val Ser Val Arg Lys Ser Val Gln Asp Asp His Ile Val Cys Leu
65 70 75 80
Glu Cys Gly Gly Ser Phe Lys Ser Leu Lys Arg His Leu Thr Thr His
85 90 95
His Ser Met Thr Pro Glu Glu Tyr Arg Glu Lys Trp Asp Leu Pro Val
100 105 110
Asp Tyr Pro Met Val Ala Pro Ala Tyr Ala Glu Ala Arg Ser Arg Leu
115 120 125
Ala Lys Glu Met Gly Leu Gly Gln Arg Arg Lys Ala Asn Arg Pro Lys
130 135 140
Lys Lys Arg Lys Val
145

<210> 28

<211> 143

<212> PRT

<213> rhizobium elti

<400> 28

Met Thr Asp Met Ala Thr Gly Asn Ala Pro Glu Leu Leu Val Glu Leu
1 5 10 15

Thr Ala Asp Ile Val Ala Ala Tyr Val Ser Asn His Val Val Pro Val
 20 25 30
 Ser Asp Leu Ala Asn Leu Ile Ser Asp Val His Ser Ala Leu Ser Asn
 35 40 45
 Thr Ser Val Pro Gln Pro Ala Ala Val Val Glu Lys Gln Lys Pro
 50 55 60
 Ala Val Ser Val Arg Lys Ser Val Gln Asp Glu Gln Ile Thr Cys Leu
 65 70 75 80
 Glu Cys Gly Gly Asn Phe Lys Ser Leu Lys Arg His Leu Met Thr His
 85 90 95
 His Ser Leu Ser Pro Glu Glu Tyr Arg Glu Lys Trp Asp Leu Pro Thr
 100 105 110
 Asp Tyr Pro Met Val Ala Pro Ala Tyr Ala Glu Ala Arg Ser Arg Leu
 115 120 125
 Ala Lys Glu Met Gly Leu Gly Gln Arg Arg Lys Arg Gly Arg Gly
 130 135 140

<210> 29

<211> 142

<212> PRT

<213> agrobacterium radiobacter

<400> 29

Met Thr Glu Thr Ala Tyr Gly Asn Ala Gln Asp Leu Leu Val Glu Leu
 1 5 10 15
 Thr Ala Asp Ile Val Ala Ala Tyr Val Ser Asn His Val Val Pro Val
 20 25 30
 Thr Glu Leu Pro Gly Leu Ile Ser Asp Val His Thr Ala Leu Ser Gly
 35 40 45
 Thr Ser Ala Pro Ala Ser Val Ala Val Asn Val Glu Lys Gln Lys Pro
 50 55 60
 Ala Val Ser Val Arg Lys Ser Val Gln Asp Asp His Ile Val Cys Leu
 65 70 75 80
 Glu Cys Gly Gly Ser Phe Lys Ser Leu Lys Arg His Leu Thr Thr His
 85 90 95
 His Ser Met Thr Pro Glu Glu Tyr Arg Glu Lys Trp Asp Leu Gln Val
 100 105 110
 Asp Tyr Pro Met Val Ala Pro Ala Tyr Ala Glu Ala Arg Ser Arg Leu

| | | |
|---|-----|-----|
| 115 | 120 | 125 |
| Ala Lys Glu Met Gly Leu Gly Gln Arg Arg Lys Ala Asn Arg | | |
| 130 | 135 | 140 |

<210> 30

<211> 143

<212> PRT

<213> rhizobium meliloti

<400> 30

| | | |
|---|-----|-----|
| Met Thr Glu Thr Ser Leu Gly Thr Ser Asn Glu Leu Leu Val Glu Leu | | |
| 1 | 5 | 10 |
| Thr Ala Glu Ile Val Ala Ala Tyr Val Ser Asn His Val Val Pro Val | | |
| 20 | 25 | 30 |
| Ala Glu Leu Pro Thr Leu Ile Ala Asp Val His Ser Ala Leu Asn Asn | | |
| 35 | 40 | 45 |
| Thr Thr Ala Pro Ala Pro Val Val Val Pro Val Glu Lys Pro Lys Pro | | |
| 50 | 55 | 60 |
| Ala Val Ser Val Arg Lys Ser Val Gln Asp Asp Gln Ile Thr Cys Leu | | |
| 65 | 70 | 75 |
| Glu Cys Gly Gly Thr Phe Lys Ser Leu Lys Arg His Leu Met Thr His | | |
| 85 | 90 | 95 |
| His Asn Leu Ser Pro Glu Glu Tyr Arg Asp Lys Trp Asp Leu Pro Ala | | |
| 100 | 105 | 110 |
| Asp Tyr Pro Met Val Ala Pro Ala Tyr Ala Glu Ala Arg Ser Arg Leu | | |
| 115 | 120 | 125 |
| Ala Lys Glu Met Gly Leu Gly Gln Arg Arg Lys Arg Arg Gly Lys | | |
| 130 | 135 | 140 |

<210> 31

<211> 16

<212> PRT

<213> Arabidopsis sp.

<400> 31

| |
|---|
| Arg Ile Glu Asn Thr Thr Asn Arg Gln Val Thr Phe Cys Lys Arg Arg |
| 1 5 10 15 |

<210> 32

<211> 18

<212> PRT

<213> Nicotiana sp.

<400> 32

Arg Arg Leu Ala Gln Asn Arg Glu Ala Ala Arg Lys Ser Arg Leu Arg
1 5 10 15

Lys Lys

<210> 33

<211> 21

<212> PRT

<213> Nicotiana sp.

<400> 33

Lys Lys Arg Ala Arg Leu Val Arg Asn Arg Glu Ser Ala Gln Leu Ser
1 5 10 15

Arg Gln Arg Lys Lys
20

<210> 34

<211> 18

<212> PRT

<213> Zea sp.

<400> 34

Arg Lys Arg Lys Glu Ser Asn Arg Glu Ser Ala Arg Arg Ser Arg Tyr
1 5 10 15

Arg Lys

<210> 35

<211> 47

<212> PRT

<213> Unknown

<220>

<223> potyvirus nuclear localization signal

<220>

<221> x

<222> (12)..(44)

<223> unknown or other amino acid

<400> 35

Lys Lys Asn Gln Lys His Lys Leu Lys Ala Ala Met Xaa Xaa Xaa Xaa
1 5 10 15

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa
20 25 30

Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Xaa Lys Arg Lys
35 40 45

<210> 36

<211> 17

<212> PRT

<213> Xenopus sp.

<400> 36

Lys Arg Pro Ala Ala Thr Lys Lys Ala Gly Gln Ala Lys Lys Lys Lys
1 5 10 15

Leu

<210> 37

<211> 17

<212> PRT

<213> Xenopus sp.

<400> 37

Lys Arg Ile Ala Pro Asp Ser Ala Ser Lys Val Pro Arg Lys Lys Thr
1 5 10 15

Arg

<210> 38

<211> 17

<212> PRT

<213> Xenopus sp.

<400> 38

Lys Arg Lys Thr Glu Glu Glu Ser Pro Leu Lys Asp Lys Asp Ala Lys
1 5 10 15

Lys

<210> 39

<211> 17

<212> PRT

<213> Mus sp./Rattus sp.

<400> 39

Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys
1 5 10 15

Lys

<210> 40

<211> 17

<212> PRT

<213> Homo sapiens

<400> 40

Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys
1 5 10 15

Lys

<210> 41

<211> 17

<212> PRT

<213> Homo sapiens

<400> 41

Arg Lys Cys Leu Gln Ala Gly Met Asn Leu Glu Ala Arg Lys Thr Lys
1 5 10 15

Lys

<210> 42

<211> 17

<212> PRT

<213> Gallus sp.

<400> 42

Arg Lys Cys Cys Gln Ala Gly Met Val Leu Gly Gly Arg Lys Phe Lys
1 5 10 15

Lys

<210> 43

<211> 17

<212> PRT

<213> Homo sapiens

<400> 43

Arg Lys Cys Tyr Glu Ala Gly Met Thr Leu Gly Ala Arg Lys Leu Lys

Lys

<211> 17

<212> PRT

<213> Gallus sp.

<400> 44

Arg Arg Cys Phe Glu Val Arg Val Cys Ala Cys Pro Gly Arg Asp Arg
1 5 10 15

Lys